
Worms and fish...
more than just bait
Kathryn Hall

# Gyliauchenidae

## Goto & Matsudaira, 1918

- digenean parasites (?) of rectum
- restricted to herbivorous reef fishes:

  Siganidae, Acanthuridae, Zanclidae, Scaridae, Pomacanthidae, Chaetodontidae
- found only in Pacific and Indian Oceans
- diagnosed by oesophageal bulb, posterior ventral sucker

**So why should we be interested in the worms from the rectum of fishes?**

# The vibrant community

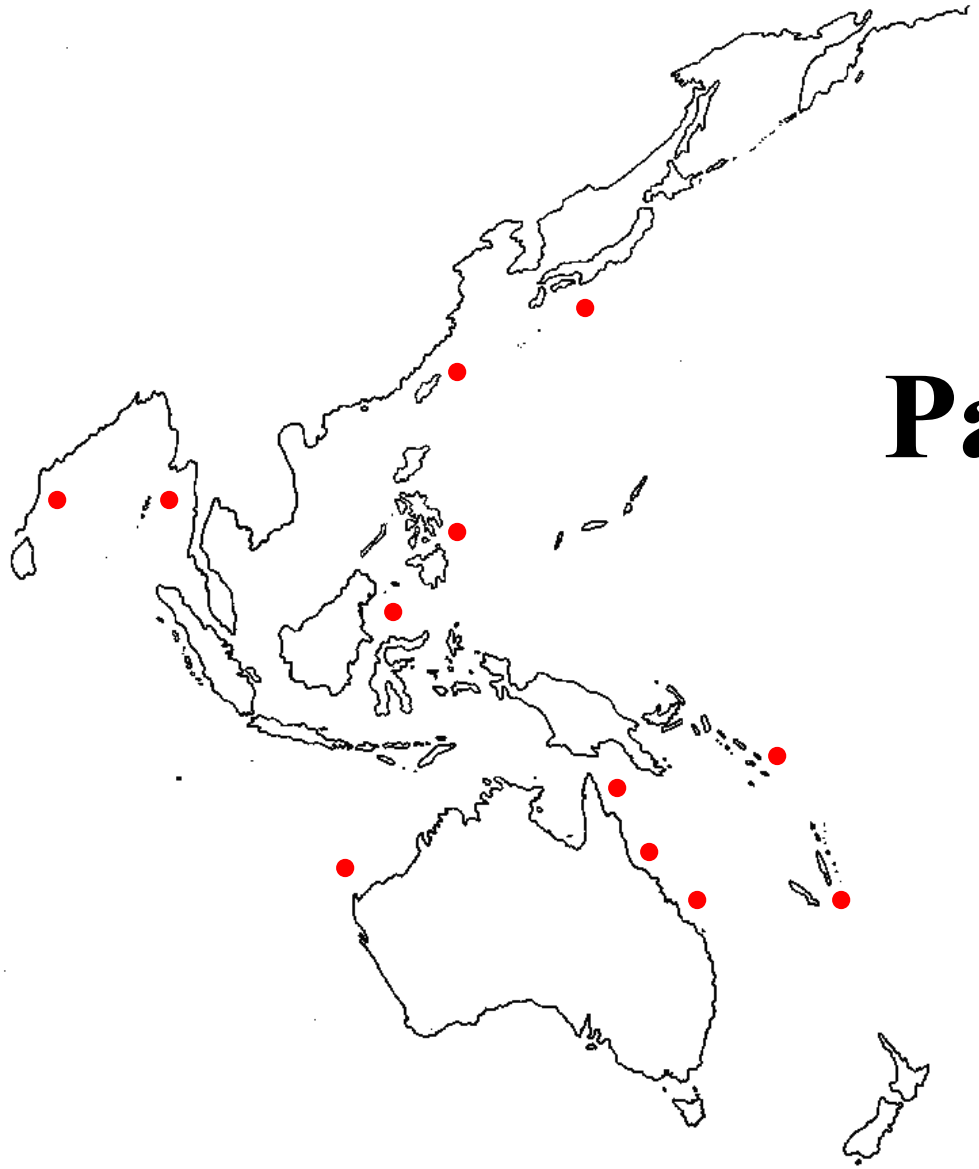
- fermentative digestion (?)
- bacteria:
  the world's largest bacterium found in *Acanthurus* spp.
- ciliates
- Digenea:
  *Hexangium* (Angiodictyidae)
  gyliauchenids
- ectocommensal microbes of digeneans

# Taxonomy 1995

- relatively small family
- 7 genera recognised by Nahhas & Wetzel:

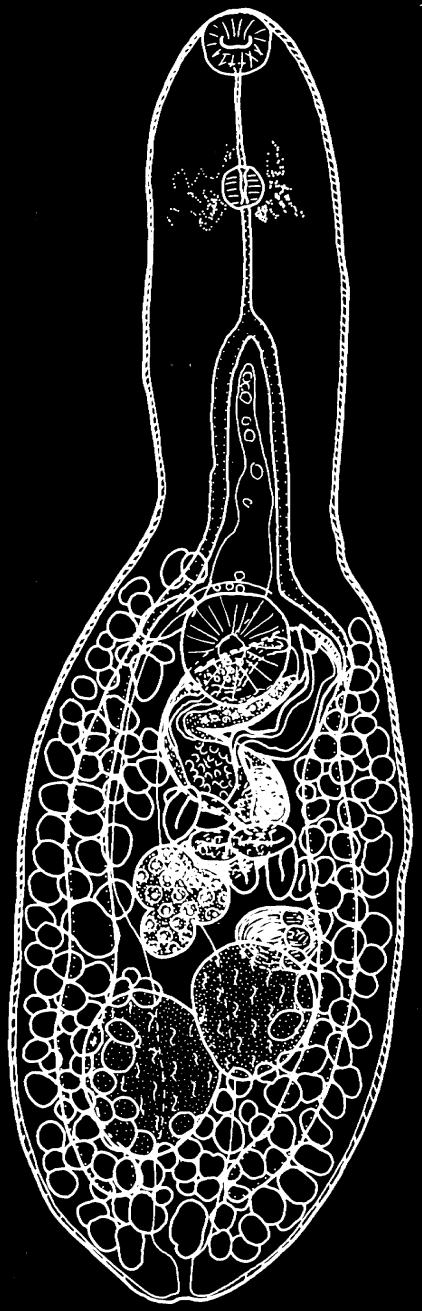
  ***Gyliauchen* (11 spp.)**
  ***Flagellotrema* (4 spp.)**
  ***Ichthyotrema* (1 sp.)**
  ***Leptobulbus* (1 sp.)**
  ***Apharyngogyliauchen* (3 sp.)**
  ***Paragyliauchen* (2 sp.)**

- Total number = 22 spp.



# Pacific Ocean

• Distribution of Gyliauchenidae



# Progress as of 1995

- broadly restricted to herbivorous reef fishes
- absent from Atlantic fishes

  = restriction to Indo-West Pacific reefs
- phylogenetic links with Lepocreadiidae (Blair & Barker, 1993)

# Problems as of 1995

- *Flagellotrema* = *Gyliauchen*
- *Petalocotyle* not recognised
- *Robphildollfusium* (Atlantic) not recognised
- *Gyliauchen* spp. poorly defined:
  difficulties in ID lead to spurious records
- poor specimen preparation = no ID
- interrelationships of genera???

# Aims

- investigate biodiversity on GBR
- revise taxonomy:
  - independent generic classification
  - incorporate species from GBR
- elucidate phylogeny:
  - character evolution
  - historical biogeography
  - co-evolution?

→ small size makes this seem do-able

# From boxes to slides via islands

- boxes of vials on Tom's shelf
- Renae East (Honours 1996-7)
- my collecting
- Clinton Chambers' collecting
- kind donations

= 2000 slides (!!!)

# Fruits of collecting

| Family | No. examined | No. positive | |
|---|---|---|---|
| Kyphosidae | 7 | 0 | (0.0%) |
| Chaetodontidae | 1291 | 7 | (0.5%) |
| Zanclidae | 38 | 8 | (21.1%) |
| Acanthuridae | 784 | 196 | (25.0%) |
| Scaridae | 109 | 34 | (31.2%) |
| Pomacanthidae | 94 | 38 | (40.4%) |
| Siganidae | 312 | 246 | (78.8%) |
| TOTAL | 2635 | 518 | (19.7%) |

# Biodiversity uncovered

| Host | Gyliauchenidae |
|---|---|
| Scaridae | 1 sp. |
| Pomacanthidae | 2 spp. |
| Zanclidae | 2 spp. |
| Acanthuridae | 13 spp. |
| Siganidae | 21 spp. |
| TOTAL | 39 spp. |

# New status of Gyliauchenidae

- recognition of *Petalocotyle*
- new total number of genera = 10:
  *Petalocotyle* (3 spp.)
  *Progyliauchen* (1 sp.)
  *Affecauda* (1 sp.)
- new total number of species = 61 spp.
- distribution still restricted to Indo-Pacific

# New species, but which genus?

- current genera inadequate
- distinct forms siphoned off to leave one dumping-ground taxon: *Gyliauchen*
- overlapping diagnoses
- no synapomorphies
- character “contamination”
- few stable characters

# Generic solutions

- solution needs to be based on phylogeny
- no evolutionary taxonomy or cladistics
- only 1 gyliauchenid sequence (28S D1)
- most obvious character regarded as too variable for taxonomy (Ozaki, 1933)

→ time to sequence!

# Gylis go genetic

- test my OTUs and elucidate phylogeny
- 4 genes chosen:

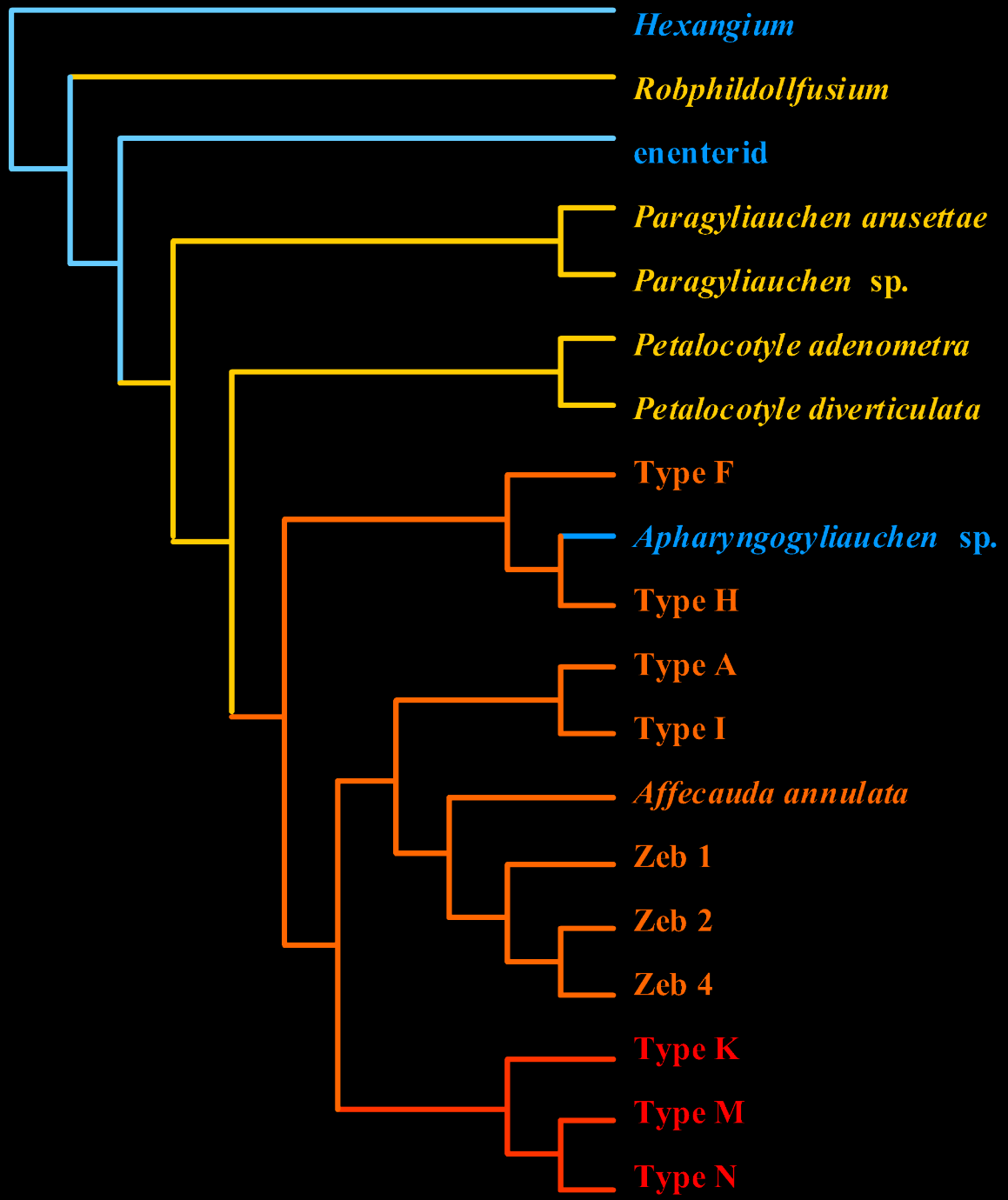
| | |
|---|---|
| 16S mt rDNA (~600 bp) | 8 OTUs |
| ND1 mt DNA (594 bp) | 19 OTUs |
| 28S rDNA (D1-D4) (~1200 bp) | 19 OTUs |
| ITS2 rDNA (~600 bp) | 19 OTUs |

- replicates for location and host combinations

# Pounding at PAUP*

- lepocreadiids and others as outgroups
- ND1 data incongruent with 28S and ITS2
- simultaneous analysis yielded same results as combined analysis (no taxonomic incongruence)
- differential weighting of protein sequence did not alter topology
- 1 optimal phylogeny from simultaneous analysis

# Puzzle consensus based on ND1, ITS2 & 28S genes



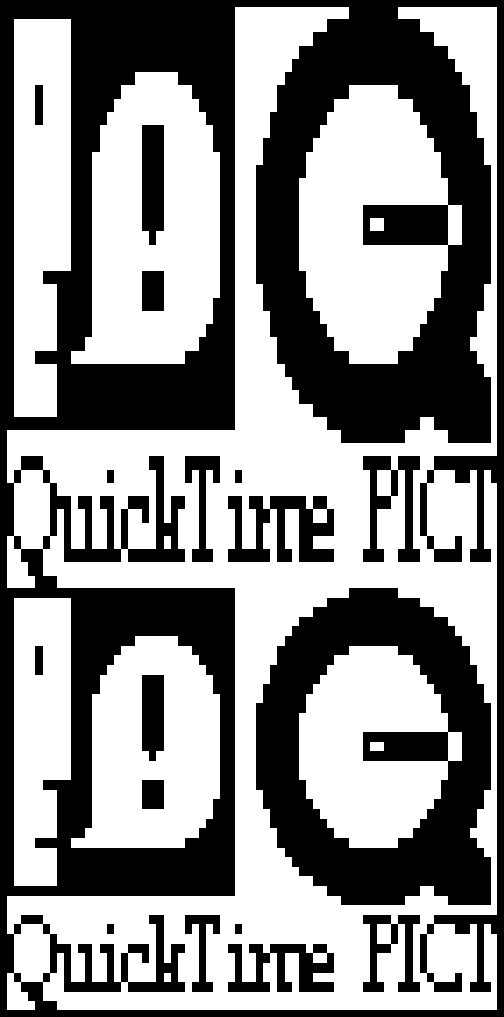
# Phylogeny for history's sake?

- gut becomes more complex through time
- oesophagus structure stable and heritable
- hierarchy of synapomorphies for gyliauchenid clades
- heritability of characters has taxonomic implications

→ genera can now be defined

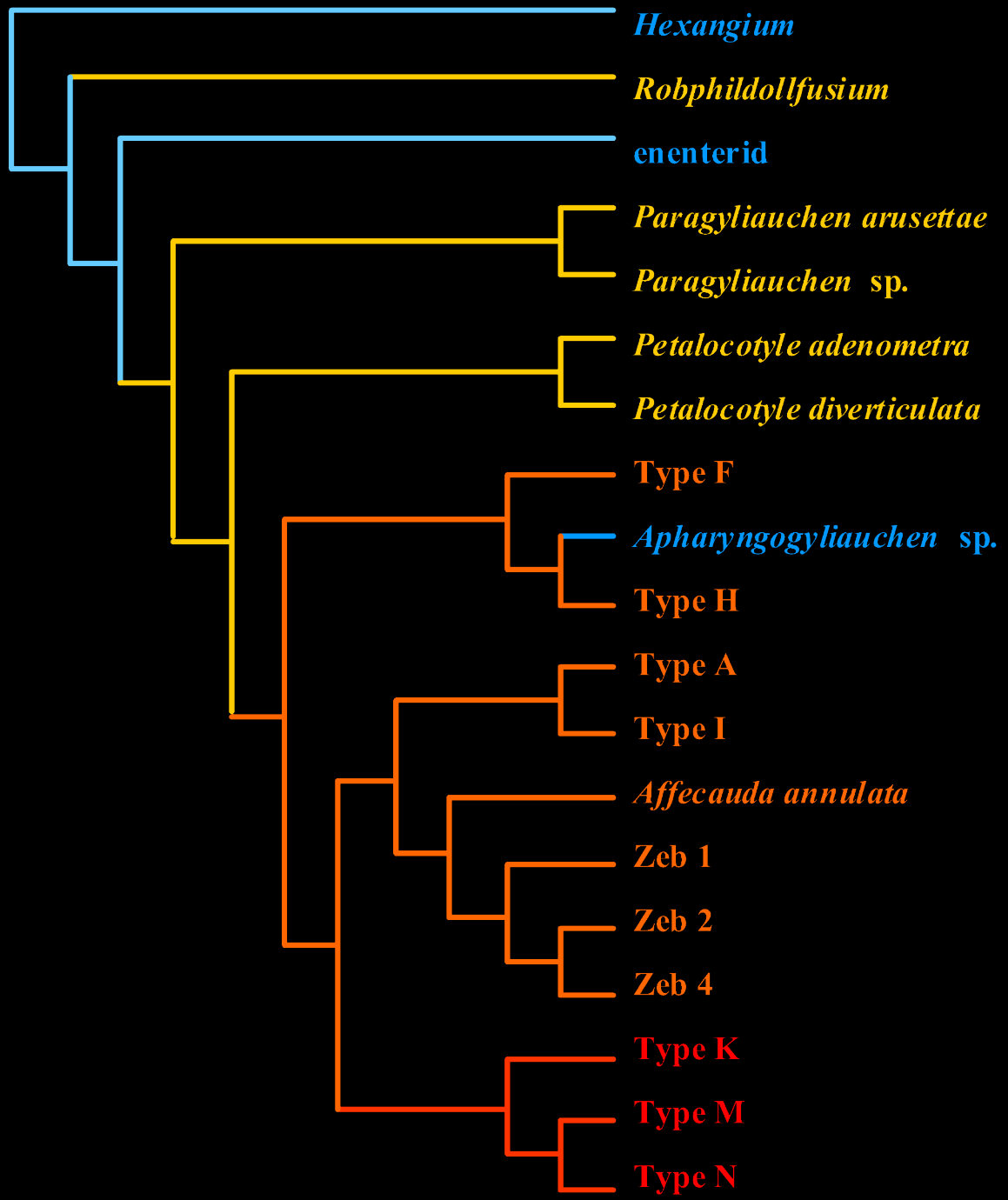
*Paragyliauchen*

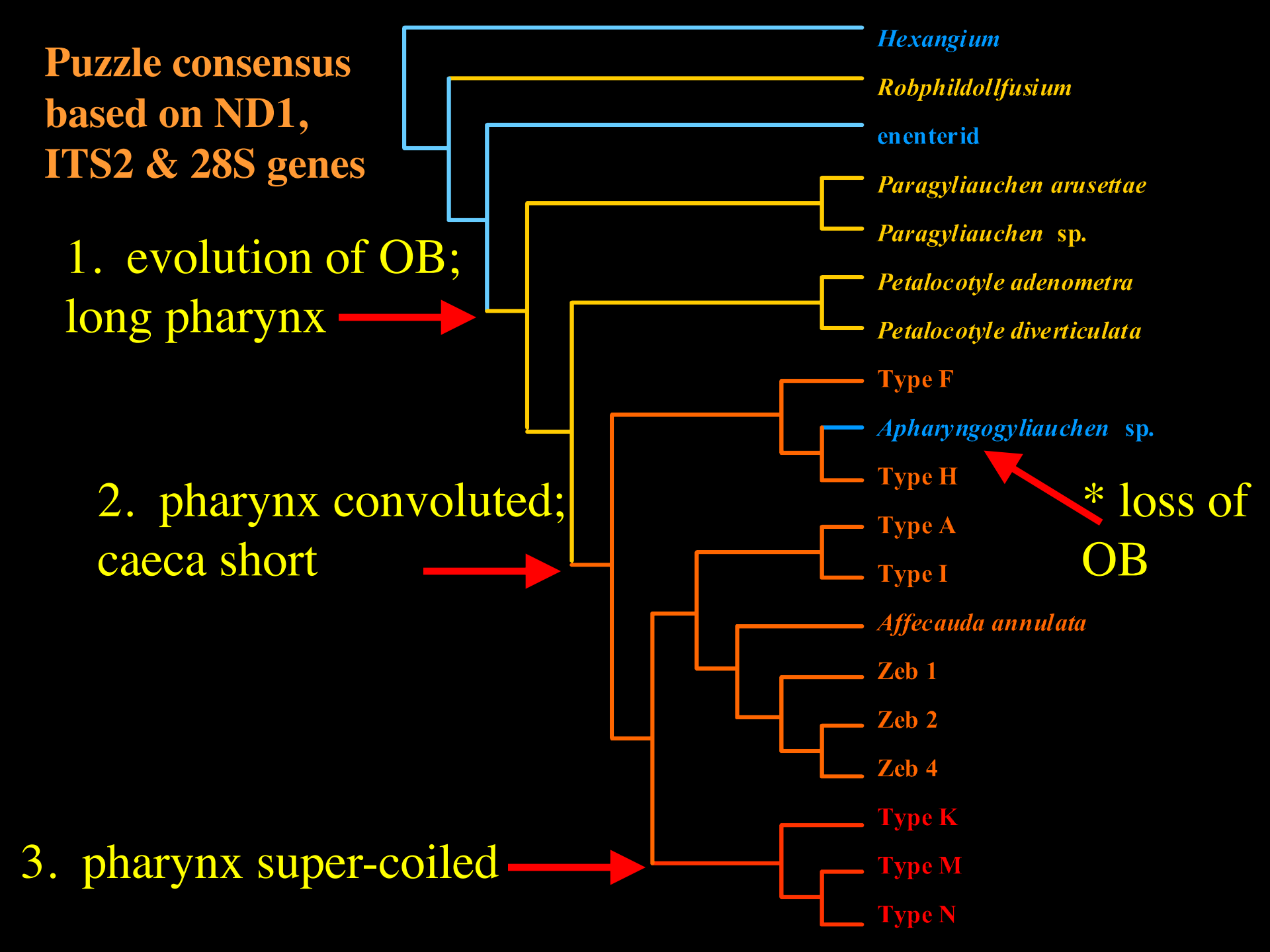
Type A



Type M

# Puzzle consensus based on ND1, ITS2 & 28S genes




Puzzle consensus based on ND1, ITS2 & 28S genes
1. evolution of OB; long pharynx
2. pharynx convoluted; caeca short
3. pharynx super-coiled
* loss of OB
Hexangium
Robphildollfusium
enenterid
Paragyliauchen arusettae
Paragyliauchen sp.
Petalocotyle adenometra
Petalocotyle diverticulata
Type F
Apharyngogyliauchen sp.
Type H
Type A
Type I
Affecauda annulata
Zeb 1
Zeb 2
Zeb 4
Type K
Type M
Type N

# Consequences and caveats

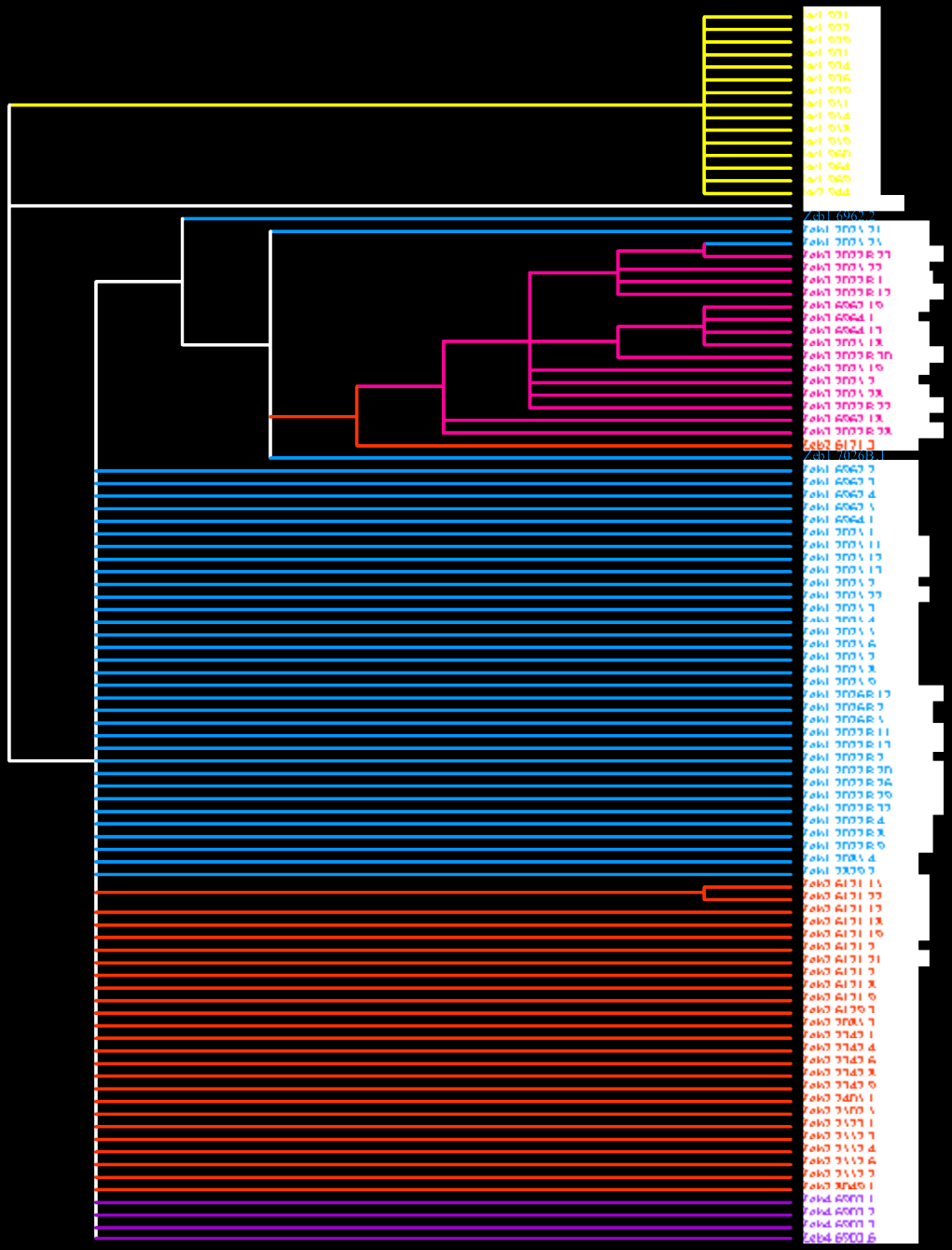
- only 14 gyliauchenid OTUs on gene trees
- 25 OTUs untested by cladistics
- some new samples remain to be sequenced
- remainder must be incorporated using other techniques
- morphometrics?

# Morphometric nightmares

- traditionally the playground of pheneticists
- multivariate statistics successfully used by helminthologists for species discrimination
- cladists dipped in their toes with gap coding
- Thiele (1993) pioneered gap-weighting for botanical data
- can these methods work for worms?

# To Ln(x) or length standardise?

- 6 OTUs tested by comparison with *Paragyliauchen arusettae*
- Ln (body length)
- 15 other variables length standardised
- discretised as multistate characters
- heuristic parsimony analysis
- (tentative) resolution



Israel 1

Zebrasoma 2

Zebrasoma 1

Zebrasoma 3

Zebrasoma 4

# Morphometrics: MVA or PAUP*?

- jury still out
- PCA and cluster observations do work at specific level
- parsimony analysis does seem to have some resolving power

→ need to do a larger matrix with more taxa

# Kathryn: Quo vadit?

- finalise taxonomy: drawings and generic revision
- sequence new collections
- sequence hosts
- co-evolution?